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BIOLOGY AND MANAGEMENT OF *LONGIDORUS AMERICANUS* IN A SOUTHERN USA NURSERY

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ABSTRACT

A recently discovered nematode, *Longidorus americanus*, caused stunting of *Pinus taeda* seedlings at a forest-tree nursery in Georgia, USA. In growth chamber experiments, *L. americanus* significantly reduced the root size of *P. taeda* and *P. elliottii* seedlings. Although the root systems of *P. palustris* were not significantly reduced, it was found to be a host for *L. americanus*. The field where damage by *L. americanus* occurred was in continuous production of *Pinus* spp. and *Quercus alba* seedlings from 1990 to 1998. Fumigation by MC33 (methyl bromide 67%/chloropicrin 33%) in the spring of 1998 and again in 2000 remedied the problem for only one year; the seedlings were severely stunted during the second production years (1999 and 2001). Growth chamber tests found that *Quercus* spp. (*Q. alba, Q. rubra, Q. falcata, Q. virginiana,* and *Q. nigra*) were hosts for *L. americanus*. The populations of nematodes declined with *Q. acutissima*. Grasses used as cover crops in southern (USA) nurseries, including *Triticum aestivum*, *Secale cereale*, *Sorghum bicolor*, *Avena sativa* and *Panicum ramosum* are not hosts for *L. americanus*. Weeds such as *Cyperus esculentus* and *C. rotundus* that are common in southern (USA) nurseries were also not suitable hosts for the nematode. A fallow study conducted in a field and in growth chambers suggests that *L. americanus* does not survive in soil for extended periods (> 334 days) without a suitable host. Surveys for *L. americanus* have determined that the nematode occurs in oak forests that border the nursery and in an adjacent pine seed orchard.

Key words: *Longidorus americanus, Pinus* sp., *Quercus* sp., cover crops, *Cyperus* sp. fallow

INTRODUCTION

A new species of *Longidorus* discovered in 2000 was associated with stunting of *Pinus taeda* in a forest nursery located in southern Georgia, USA (FRAEDRICH, CRAM 2002). The nematode was recently described and named *Longidorus americanus* (HANDOO et al. 2005). Symptoms of seedling damage by *L. americanus* include chlorotic needles, reduced shoot growth and root systems that lack lateral and fine roots. The stunted seedlings were found in field 7S where similar damage had been observed since 1996 on both *P. taeda* and *P. elliottii* crops. The cause of this damage went undiagnosed until 2000 largely due to our reliance on a nematode testing laboratory that processed soil using a sugar flotation method. This technique is more suitable for smaller plant-parasitic nematodes. The adult nematodes of *L. americanus* are large (7 - 8 mm long) and require extraction methods specific for large nematodes (FLEGG 1967, SHURTLEFF, AVERRE 2000). Over the next 5 years, a series of surveys, dosage response tests, host range tests, and fumigation and fallow studies were conducted to investigate various aspects of the biology of the nematode and determine how best to manage this problem. In all our surveys and studies, nematodes were extracted using a technique developed by FLEGG (1967) with minor modifications by FRAEDRICH and CRAM (2002).

Tab. 1.
Field history of fumigation and crop rotations of pine, hardwoods, and cover crops for blocks 1 - 5 in field 7S of a south Georgia forest tree nursery (Byromville GA).

Date	Block 1[+]	Block 2	Block 3	Block 4	Block 5
1989	**MC33**[‡]	**MC33**	**MC33**	**MC33**	**MC33**
1990	*P. taeda*[*]	*P. taeda*	*P. taeda*	*P. taeda*	*P. taeda*
1991	*P. taeda*	*P. taeda*	*P. elliottii*	*P. elliottii*	*P. elliottii*
1992	*P. taeda*	*P. taeda*	*P. elliottii*	**Basamid/** *P. elliottii*	**MC33/** *P. elliottii*
1993	*Q. alba|P. taeda*	*P. taeda*	**Fallow**	**Fallow/ MC33**	**Fallow/ MC33**
1994	*Q. alba*	*Q. alba*	*C. florida|L. styraciflua*	*P. elliottii*	*P. elliottii*
1995	*Q. alba*	*Q. alba*	*C. florida|L. styraciflua*	*P. virginiana*	*P. virginiana|P. clausa*
1996	***P. taeda***|*Q. alba*	*Q. alba*	*L. bicolor*	*L. bicolor*	*S. cereale*
1997	***P. taeda***	*P. taeda*	*L. bicolor*	*S. bicolor*	*S. bicolor*
1998	**Fallow**	**MC33/** *P. elliottii*	**MC33/** *P. elliottii*	**Fumigation study/** *P. taeda*	**Fumigation study/** *P. taeda*
1999	**Fallow**	***P. taeda***	***P. taeda***	*P. taeda*	*P. taeda*
2000	**MC33/** *P. taeda*	**MC33/** *P. taeda*	**MC33/** *P. taeda*	***P. taeda***	***P. taeda***
2001	***P. taeda***	***P. taeda***	***P. taeda***	***P. taeda***	***P. taeda***

[+]Text – stunted seedling damage
[‡]MC33 = methyl bromide 67% / chloropicrin 33%
[*]*P. taeda = Pinus taeda; P. elliottii = Pinus elliottii; Q. alba = Quercus alba; P. virginiana = Pinus virginiana; P. clausa = Pinus clausa; C. florida = Cornus florida; L. bicolor = Lespedeza bicolor; L styraciflua = Liquidambar styraciflua; S. cereale = Secale cereale*

FIELD HISTORY

The crop and fumigation history of field 7S, provided in Table 1, is vital to understanding the development of the problem caused by *L. americanus*. Blocks 1 and 2 of this field were used to test rotations of pine and white oak from 1990 to 1996. This crop rotation was experimental and was not part of the normal crop rotation used by this nursery or other southeastern forest-tree nurseries. In 1996 and again in 1997, a few areas of stunted *Pinus taeda* seedlings occurred in block 1. Seedlings were examined and soil was sent to a nematode laboratory, but these efforts failed to find a cause for the damage. Blocks 2 and 3 in field 7S were fumigated in 1998 with 67% methyl bromide/33% chloropicrin (MC33) and planted to *P. elliottii*. Block 1 was too wet at the time of fumigation and remained fallow during 1998. A fumigation study was also established in blocks 4 and 5, and sown with *P. taeda* (CRAM et al. 2002). In 1999, large areas of stunted seedlings occurred in block 2 and extended into block 3 (Fig. 1). Seedlings from the affected areas were examined for fungi and soil samples were sent to a nematode testing laboratory, but again the cause of the damage could not be identified. Blocks 1 to 3 were fumigated with MC33 in the spring of 2000 and sown in *P. taeda.*

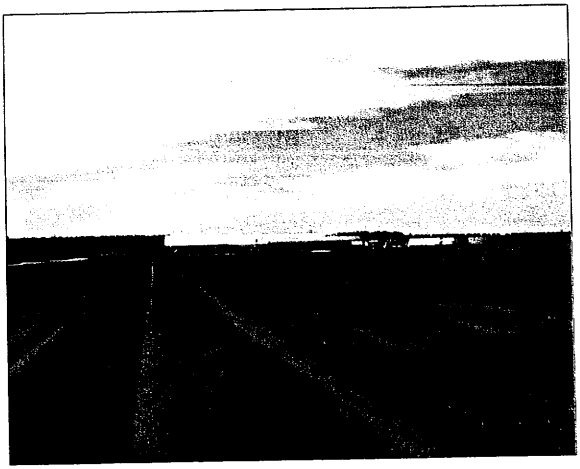

Fig. 1.
Stunted *Pinus taeda* in block 2 of field 7S, summer 1999

In 2000, small spots (3 - 9 m long) of stunted seedlings occurred in unfumigated control plots of the fumigation study in blocks 4 and 5 (FRAEDRICH, CRAM 2002). Soil samples were sent to a nematode lab, which reported low levels of *Pratylenchus* sp. and *Criconemella* sp. Evaluations for pathogenic fungi were also inconclusive. Finally, we discovered large (5.4 - 9 mm long) plant-parasitic nematodes associated with stunted seedlings as we were examining unwashed roots; these nematodes were determined to be members of the genus *Longidorus*. We believe that the nematode was moved into these nonfumigated control plots during routine field operations before establishment of the study in 1998.

SURVEYS

1 Nursery blocks

In August and October of 2000, the populations of *L. americanus* were greatest in soil from the centers and margins of stunted areas compared to adjacent areas with healthy seedlings (FRAEDRICH, CRAM 2002). In August, 26 *L. americanus* per 25 g soil were obtained from the center of patches, 13.1 at the margins, and 0.5 and 0.2 at distances of 1.5 and 3 m from the margins, respectively. In October, there was an average of 25.8 *L. americanus* per 25 g of soil at the margins of patches, and only 1.5 *L. americanus* /25 g soil at locations 1.5 m outside the margin.

2 Outside the nursery field

Surveys were also performed from 2001 to 2003 in various locations within the nursery and in locations that border the nursery. These samples typically consisted of 8 - 10 cores at a 6" depth taken at specific locations and along transects. Soil samples were taken in 3 red cedar windrows, an oak (*Quercus* spp.) seed orchard, and oak and pine forests adjacent to the nursery. Sixteen composite soil samples were also obtained from *P. taeda* and *P. elliottii* seed orchards that border the nursery. *Longidorus americanus* was found in 37% soil samples from the pine seed orchards, and in an area of oak (primarily *Q. nigra*) trees that bordered the nursery.

Tab. 2.
Longidorus americanus population densities and root dry weights of southern pine species 26 weeks after infestation (experiment 2)+

Pine species	Initial needle nematode	Final needle nematode	Root dry ‡ weight (g)
	Number / container		
Pinus taeda	200	1257	0.159a
	0	0	0.295b
Pinus elliottii	200	1683	0.334a
	0	0	0.556b
Pinus palustris	200	820	0.681a
	0	3	0.825a

+Data obtained from FRAEDRICH et al. 2003 (Plant Dis., 87:1129:1132)
‡Means followed by the same letter do not differ significantly ($P \leq 0.05$) according to t-test

Tab. 3.
Mean number of *Longidorus americanus* obtained from soil and roots of plant species 13 weeks after infestation with 100 nematodes/container

Plant species	Longidorus sp./400cc soil+	Total estimated Longidorus sp. per container
Pinus taeda	74a	295
Quercus nigra‡	94a	377
Q. rubra	56ab	223
Q. akba‡	42ab	168
Q. falcata	33ab	131
Q. virginiana	32ab	129
Q. acutissima	17bc	69
Fallow	1c	5

+Means followed by the same letter do not differ significantly (alpha = 0.05) according to Tukey's HSD test. Square root transformation of nematode counts performed before analysis. Data analyzed as a randomized complete block design.
‡Means based on three replications

HOST RANGE STUDIES

1 Pine hosts

Population densities of *Longidorus americanus* increased on roots of *P. taeda* seedlings and damaged the root systems of seedlings in growth chamber tests. Root dry weights of seedlings decreased with respect to both the initial *L. americanus* dose, and the final population per container (FRAEDRICH, CRAM 2002). *Pinus elliottii* and *P. palustris* were also found to be hosts of *L. americanus* (FRAEDRICH et al. 2003). Population densities of *L. americanus* increased on roots of *P. elliottii* and *P. palustris*, but at the initial population densities used in these experiments only the root dry weights of *P. elliottii* seedlings were reduced by the nematode (Table 2). This lack of effect of the nematode on *P. palustris* may be related to the unique developmental characteristics of this pine species. *Pinus palustris* typically remains in a "grass stage" during the first 1.5 or more years of its development, and root system growth is favored during this period.

2 Cover crops and weeds

Small grain cover crops are typically alternated with pine and hardwood seedling production by many nurseries in the southern USA. The suitability of cover crops and weeds as hosts for *L. americanus* were evaluated in a series of experiments (FRAEDRICH et al. 2003). The species (cultivars) of cover crops tested were *Triticum aestivum* (Saluda) *Secale cereale* (Wrens Albruzzi), *sorghum bicolor* (Richardson 9300, SG Ultra), *Panicum ramosum* (DW-01), and *Avena sativa* (FLA 501). The weeds tested as hosts for *L. americanus* were *Cyperus esculentus* and *C. rotundus*. *Longidorus americanus* population densities decreased substantially on roots of all cover crops and weeds, and were not significantly different than populations in the fallow containers (FRAEDRICH et al. 2003).

3 Oak host range

Six species of oak have been evaluated as hosts for the *L. americanus* (unpublished data). The oak species tested were *Quercus virginiana*, *Q. acutissima*, *Q. alba*, *Q. nigra*, *Q. falcata*, and *Q. rubra*. *Pinus taeda* and fallow treatments were also included. Soil (loamy sand) from the nursery field was microwaved for 8 minutes in 2,000 g batches, and containers were filled with 1,600 cc of soil. There were four replications (containers) of each species, and germinated oak and loblolly pine seeds were established in their respective containers. Fallow containers were maintained free of all plants. The containers were infested with 100 adult and juvenile nematodes when the oaks were 15-week old and the pines were 7-week old. Containers were placed in growth chambers at 25 °C with a 14 hr photoperiod. After 13 weeks, *L. americanus* population densities had increased on roots of *Q. virginiana*, *Q. alba*, *Q. nigra*, *Q. falcata*, and *Q. rubra* (Table 3). *Quercus acutissima* was the only species that had significantly less nematodes than loblolly pine, and the population density did not differ significantly from the fallow treatment.

FALLOW STUDIES

Several nurseries in the southern USA have begun to alternate tree seedling production with summer fallow in order to control nutsedge with glyphosate (FRAEDRICH et al. 2003). The effect of fallow on the survival of *L. americanus* was determined in field and growth chamber studies during 2002 (FRAEDRICH et al. 2005). In the field study, the population density of *L. americanus* decreased steadily during the first 101 days in the fallow field, and only a few nematodes were detected between 128 to 220 days (Figure 2; $P \leq 0.0001$). *Longidorus americanus* was not detected in soil samples from any plot on days 263 (January), 325 (March) or 365 (May).

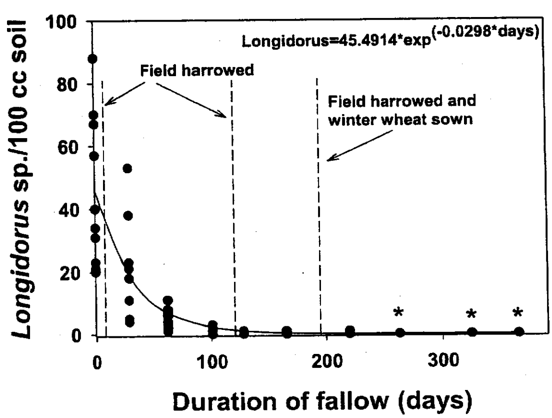

Fig. 2.
Relationship between *Longidorus americanus* population densities and days of fallow in field plots after April 11, 2002
Asterisks (*) at sample days indicate that the needle nematode was not detected in any field plot (FRAEDRICH et al. 2005).

In the growth chamber study, the population of *L. americanus* also decreased exponentially over time ($P \leq 0.0001$) in fallow containers (FRAEDRICH et al. 2005 in press). *Longidorus americanus* was not detected after days 334 and 427. In containers planted with pine seedlings, the population density of *L. americanus* initially declined but subsequently increased after additional pine seedlings were added to containers. *Longidorus americanus* does not survive for extended periods in the upper 15 cm of fallow soil in the field (> 263 days) or in fallow containers under optimal conditions (> 334 days). The use of fallow or use of non-host cover crops should provide control of the *L. americanus* nematode in the southern Georgia nursery.

DISCUSSION AND CONCLUSIONS

Longidorus species have been found in southern pine nurseries and forests (HOPPER 1958, RUEHLE, SASSER 1962); however, there have been no reports of these nematodes damaging pine seedlings in nursery beds. In 2000, *Longidorus americanus* was discovered to cause stunting of *Pinus taeda* seedling at a south Georgia forest nursery (FRAEDRICH, CRAM 2002). *Pinus elliottii* and *P. palustris* were found to be hosts of *L. americanus* (FRAEDRICH et al. 2003).

Damage by *L. americanus* occurred in a field where the nursery had alternated *P. taeda* production with production of *Quercus alba*. Host suitability tests have found that *Q. alba* and other native oak species are hosts for *L. americanus*. The initial development of pine seedling stunting in block 1 of field 7S was most likely due to continuous cropping of pine and oak species over an extended period of time.

Surveys have found *L. americanus* outside the nursery under *Q. nigra* trees (FRAEDRICH, unpublished) and in an adjacent *P. taeda* and *P. elliottii* seed orchards. Locations such as these may provide the sources for reintroduction in nursery fields through soil and water movement (e. g. floods, equipment, wind and animals). It is possible that this nematode has been introduced to the nursery fields in the past, but that the typical rotations of tree seedling crops with grass cover crops has provided adequate control of this nematode.

In 1998 and 2000, the nursery attempted to remedy the problem of stunting in pine seedling crops by using methyl bromide fumigation. Fumigation suppressed disease development in the first seedling crop after fumigation, but the problem reappeared during the second year of production. Although fumigation has been found to depress high nematode populations (DROPKIN 1989), nematode populations can rebound quickly and significantly impact subsequent seedling crops (MCKENRY, THOMASON 1976). The rebound of nematode populations after fumigation was demonstrated by FRAEDRICH and DWINELL (2003). In this study, dazomet and metam sodium reduced the needle nematode to nondeductible levels in the upper 15 cm of soil but populations subsequently increased during loblolly pine production to levels comparable to those in control plots by the end of the growing season (FRAEDRICH, DWINELL 2003).

The nursery where the *Longidorus* problem was found typical rotates from pine production to cover crops every two years. In fact, the majority of southern nurseries rotate their tree seedling production with small grain crops yearly or biennially (CRAM, FRAEDRICH 1997). All of the small grain cover crops used at this nursery were found to be non-hosts for *L. americanus*. These results coupled with the findings that *L. americanus* does not appear to survive extended periods in fallow soil may help explain why this nematode problem has not been a reoccurring problem in other fields at the nursery. We believe that the nursery will not have a serious problem with *L. americanus* in the future if they maintain the practice of alternating cover crops and periods of fallow with pine seedling production on a regular basis.

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Diseases and insects in forest nurseries

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